

An Efficacy and Immunogenicity Study Using a Modified-Live Intranasal Bovine Coronavirus Vaccine for the Protection of Calves against Enteric Disease Caused by Bovine Coronavirus Challenge

SUMMARY

Calf diarrhea is a prevalent disease in the neonate and can be challenging to treat. One of the four major pathogenic causes of calf diarrhea is Bovine Coronavirus (BCV).¹ Prevention of disease is always more desirable than treatment. This study asked the question; can an intranasal modified-live Bovine Coronavirus vaccine provide protection against enteric disease caused by BCV?

Colostrum-deprived (CD) Holstein calves were vaccinated intranasally with a single dose of modified-live (MLV) Coronavirus vaccine between 3 and 5 days of age. Approximately 30 days later, they were shipped 100 miles to simulate the stress of transport and then subsequently challenged with BCV. After observing both vaccinates and control calves for the following 15 days, vaccinated calves showed a significant reduction in severity (total number of days) of moderate or severe enteric disease ($p=0.0009$) and a significantly shorter ($p=0.0017$) duration (days from start to finish of signs) of moderate or severe enteric disease.

INTRODUCTION

Bovine Coronavirus has long been implicated as a major cause of diarrhea and involved in the respiratory disease complex in young calves and winter dysentery in adult dairy cows.² BCV replicates readily in the mucosal tissues of the upper respiratory tract (URT) and is hypothesized to be transported to the small intestine via swallowed mucous secretions.² Mucosal vaccination via intranasal application of a modified-live Bovine Coronavirus vaccine was hypothesized to protect against enteric disease caused by a heterologous BCV challenge.

MATERIALS AND METHODS

In early 2013, healthy colostrum-deprived calves were obtained from a commercial supplier. Calves were processed at the supplier site which included iodine navel dips every four to six (4-6) hours pre-shipment and administration of Vital E[®]-A+D (Stuart products), Ecolizer[®] (anti-E.coli, Novartis), iron dextran (VetOne Pharmaceuticals) and Advocin[™] (fluoroquinolone, Zoetis) per label instructions.

The calves arrived in three (3) shipments at 2 to 3 days of age and were randomized to vaccinate or placebo control within each group. They were individually identified and ear notched for Bovine Viral Diarrhea Persistent Infection (BVD PI) status.

Each calf was housed in an individual pen of approximately 25 square feet after vaccination and were then commingled post-challenge. They were fed a commercial non-medicated milk replacer twice daily plus ad libitum complete calf starter and water.

On study day -30, 22 calves were vaccinated with 1 mL of Bovilis[®] Coronavirus in each nostril (vaccinates) and another 22 calves (controls) were vaccinated in the same manner with the placebo vaccine (identical vaccine preparation without BCV). All calves were 3 to 4 days of age at vaccination.

EXPERIMENTAL DESIGN

TREATMENT GROUP	NUMBER OF CALVES	CHALLENGE DAY 0	CHALLENGE DAY 1	AGE AT CHALLENGE
PLACEBO CONTROL	22	Oral and IN	IN	30-37 days
BOVILIS CORONAVIRUS	20*	Oral and IN	IN	30-37 days

*Two (2) calves removed after vaccination and before challenge: One (2) calf died 9 days post-vaccination from E.Coli septicemia, One (1) calf not enrolled due to recurrent lameness on days 0, 1, 8, 9, 10 15, and 20 of vaccination period

Approximately thirty (30) days following the final group of vaccinations, all calves were transported 100 miles and then immediately challenged orally and intranasally with BCV (day 0). The calves were challenged again the following day (day 1) intranasally with BCV. Blood samples were collected on days -30, -16, -1, 8, and 15 post-challenge and baseline rectal temperatures were recorded on days -2,-1, and 0-8. Fecal swabs were collected days -1, 3-8 and on days

9, 12, and 15. Calves were observed throughout the post-challenge period for clinical signs of depression, dehydration, inappetence and diarrhea. All treatments were blinded. Case definitions included moderate to severe diarrhea to monitor the enteric response to challenge. Virus shedding in fecal samples was considered positive when detected levels were $> 1 \log_{10}$ TCID₅₀/mL.

RESULTS

All calves tested negative for BVD. Two (2) calves, both from the vaccinate group, were removed from the study after vaccination but prior to challenge. One (1) calf was found dead nine (9) days after vaccination due to *E. Coli* septicemia and a second calf was removed prior to challenge due to recurrent lameness.

Enteric disease was observed in calves following administration of BCV used in the challenge model. The clinical case definition of enteric disease was two (2) or more consecutive days with moderate or severe diarrhea. In the control calves, 16 of 22 (73%)

became sick with enteric disease compared to 8 of 20 (40%) of vaccinate calves ($p=0.0461$). The duration of disease was also compared between the two (2) groups and defined as the days from start to end of moderate or severe signs of diarrhea. Thirteen (13) of 22 control calves (58%) demonstrated moderate or severe enteric disease for three (3) or more consecutive days compared to 5 of 20 of vaccinates (25%). (Figure 1)

FIGURE 1: SUMMARY OF CALVES WITH ENTERIC DISEASE AFTER CHALLENGE

TREATMENT GROUP	NUMBER OF CALVES	CALVES SICK WITH ENTERIC DISEASE*	CALVES WITH LONGER DURATION OF ENTERIC DISEASES**
PLACEBO	22	16	13
BOVILIS CORONAVIRUS	20	8	5

*Defined as a calf with moderate or severe diarrhea for two (2) or more consecutive days ($p=0.0461$)

**Defined as a calf with moderate or severe diarrhea for three (3) or more consecutive days

Severity of enteric disease was defined by counting the number of days, consecutive or not, an individual calf had moderate or severe diarrhea in the observation period after challenge. Vaccinate calves also had a significant ($p=0.0009$) reduction in the severity of moderate or severe enteric disease (2.20 days), compared to placebo control calves (4.78 days).

Total duration of disease days was defined as the number of days from when a calf first had moderate or severe diarrhea until the last day it was observed to have moderate or severe diarrhea. Vaccinate calves had a significantly ($p=0.0017$) shorter duration of moderate or severe enteric disease (3.40 days) compared to placebo control calves (6.82 days). (Figure 2)

FIGURE 2: SEVERITY AND DURATION OF ENTERIC DISEASE

TREATMENT GROUP	NUMBER OF CALVES	SEVERITY OF DISEASES (DAYS)*	DURATION OF DISEASES (DAYS)**
PLACEBO	22	4.78	6.82
BOVILIS CORONAVIRUS	20	2.20	3.40

*Severity of disease is defined as the total number of days a calf had moderate or severe diarrhea ($p=0.0009$)

**Duration of disease is defined as the total number of days a calf had moderate or severe diarrhea from the onset of clinical signs until disease resolved ($p=0.0017$)

Two (2) placebo control calves died 12 days after challenge. Both calves were dehydrated, had blood tinged contents in the colon and cecum and immunohistochemical staining on sections of the colon showed staining to BCV. Further diagnostics revealed the most likely cause of death was enteritis caused by attaching and effacing *E. coli*.

There was no significant difference in fecal BCV shedding in vaccinated calves vaccinated with Bovilis Coronavirus compared to control calves.

SEROLOGICAL RESPONSE TO BCV IN CALVES BEFORE AND AFTER CHALLENGE WITH BCV

All calves were seronegative (<64) to BCV at the time of vaccination and at 14 days post-vaccination and remained seronegative up to the day of challenge. Although vaccinates and control calves were seronegative at the time of challenge, the titer response after challenge in the vaccinated calves indicated a

strong anamnestic response to BCV. The geometric mean titer (GMT) at day 8 post-challenge was 2,454 and 129 for the vaccinates and control calves, respectively. The GMT at day 15 post-challenge was 2,456 and 270 for the vaccinates and control calves, respectively. (Figure 3)

FIGURE 3: VN ANTIBODY TITER TO BCV

TREATMENT GROUP	PRE-VAC	POST-VAC	PRE-CHALLENGE	POST-CHALLENGE	
	DAY 0	DAY 14	DAY -1	DAY 8	DAY 15
PLACEBO	Negative (Neg)	Neg	Neg	129	270
VACCINATES	Neg	Neg	Neg	2,454	2,456

CONCLUSION

The challenge model induced signs of enteric disease. A single dose of Bovilis Coronavirus administered intranasally to calves at 3 to 4 days of age demonstrated a clinical benefit by reducing the duration and severity of enteric disease signs. The mechanism of protection appears to be the result of a strong anamnestic response.

REFERENCES

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- 2) Saif LJ. Bovine Respiratory Coronavirus. *Vet Clin North Am Food Anim Pract.* 2010 Jul;26(2):349-64.